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AUTHOR(S):

KONDO, AKINORI

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The Experimental Studies on the Pathogenesis of Hemorrhagic Infarction and on the Application of Fibrinolytic Treatments for Cerebral Infarction in Dogs

by

AKINORI KONDO

From the 1st Surgical Division and Neurosurgery, Kyoto University Medical School
(Director : Prof. Dr. CHISATO ARAKI)
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INTRODUCTION

Attempts to produce experimenal cerebral infarction by occlusion of the cerebral arteries have been tried for many years. It was shown by these works that infarcted foci could be scarcely made, especially in dogs, without occlusion of the middle cerebral artery[10]. Even by the occlusion of the middle cerebral artery, the infarcted foci could not be produced constantly, and the lesions produced varied in size and location. Such a low incidence of infarction and the variation in size and location of the lesions must be ascribed to the existence of abundant and efficient collateral circulations in dogs, such as by the circle of Willis, the leptomeningeal anastomoses, and anastomoses between the internal and external ethmoidal and between internal and external ophthalmic arteries.

Thus, a method of consistent production of "definite focal cerebral softening" with a low incidence of premature death was investigated in our laboratory, and J. HANDA (1964) devised a new method of intracarotid injection of fresh clot or that of production of mural thrombi in the common carotid artery combined with the clipping of the homolateral posterior communicating and the anterior cerebral arteries in dogs. The intracranial portion of the internal carotid artery as well as the proximal 2cm segment of the middle cerebral artery were occluded in this method by a continuous strand of injected fresh clot in almost all cases, causing the infarcted foci which were uniform in size in the territory of the distal portion of the middle cerebral artery. This work demonstrates that cerebral infarction can be produced in dogs in a consistent manner when the cerebral collateral channels are restricted. The lesions produced by this method were histologically divided into the following four groups; 1) petechial hemorrhage without infarction, 2) pale (non-hemorrhagic) infarction, 3) hemorrhagic infarction and 4) massive hemorrhage. The mechanism of production of such different types of lesions, especially the hemorrhagic and pale infarctions were not clearly demonstrated. GLOBUS (1938) and GLOBUS and STRAUSS (1927) contended that hemorrhagic and non-hemorrhagic infarctions were merely different stages of the same process, the terminal phase of which was massive cerebral hemorrhage. On the other hand, HAIN et al. (1952) emphasized that hemorrhagic or non-hemorrhagic infarctions could be resulted in dogs mainly by the site of occlusion of the middle cerebral artery, distal to or within the zone of the origin

of the perforating ganglionic branches.

The present study, therefore, was intended in dogs to elucidate the mechanism of hemorrhagic or non-hemorrhagic infarction by restricting the cerebral collateral channels more strictly, remaining only the middle cerebral artery which supplied the basal ganglia. After that, the effects of the fibrinolytic treatments on the lesions produced by these methods were investigated.

MATERIALS AND METHODS

Adult mongrel dogs, weighing 8 to 12kg, unselected to sex and age, were used. The dogs were anesthetized by intravenous pentobarbital sodium in an initial dose of 30 mg per body weight, supplemented as much as necessary. With the dog in the right recumbent position, the left subtemporal craniectomy was performed, resecting the zygomatic arch partially to allow the better access to the base of the skull. The dura mater was incised and the piriform lobe was gently retracted to expose the circle of Willis.

After that, the following 6 kinds of experiments were performed:

PART I : Occlusion of the middle cerebral artery proximally and distally
PART II : Measurement of the intraluminal pressure of the middle cerebral artery
PART III : Estimation of the tissue plasminogen activators
PART IV : Vital staining methods of the brain tissue by trypan blue
PART V : Quantitative measurements of the radioactivities of the infarcts
PART VI : Application of fibrinolytic enzymes for the cerebral infarction

PART I

As the preliminary study, the following experiments were carried out to investigate how the character of the lesions vary depending on the site of occlusion of the middle cerebral artery and/or the development of collateral circulation. The middle cerebral artery was clipped proximally (from its origin to the site of the perforating branches) or distally (from the origin of the perforating branches to the first major cortical bifurcation).

In this group, the dogs were sacrificed 1 week after the operation and the brains were fixed in 10% formalin saline for about 1 week and sectioned to be examined macroscopically and microscopically.

RESULTS

When the clipping of the middle cerebral artery proximal to the origin of the perforating branches was carried out, infarcted foci were found both in the thalamus and basal ganglia in all ten experimental animals. Seven of cases of them were hemorrhagic and the remaining three ischemic macroscopically.

On the other hand, when the middle cerebral artery was clipped at the distal portion to the perforating arteries before branching of the first major cortical bifurcation, infarctions were noted in the basal ganglia as well as in the thalamus in seven out of the nine experimental animals. Six of them were anemic, one hemorrhagic and the remaining two had no infarction (Table 1). The location and the property of the infarcted foci which were produced in the two groups are shown in Fig. 1-a, b. Fig. 2-a shows a hemorrha-

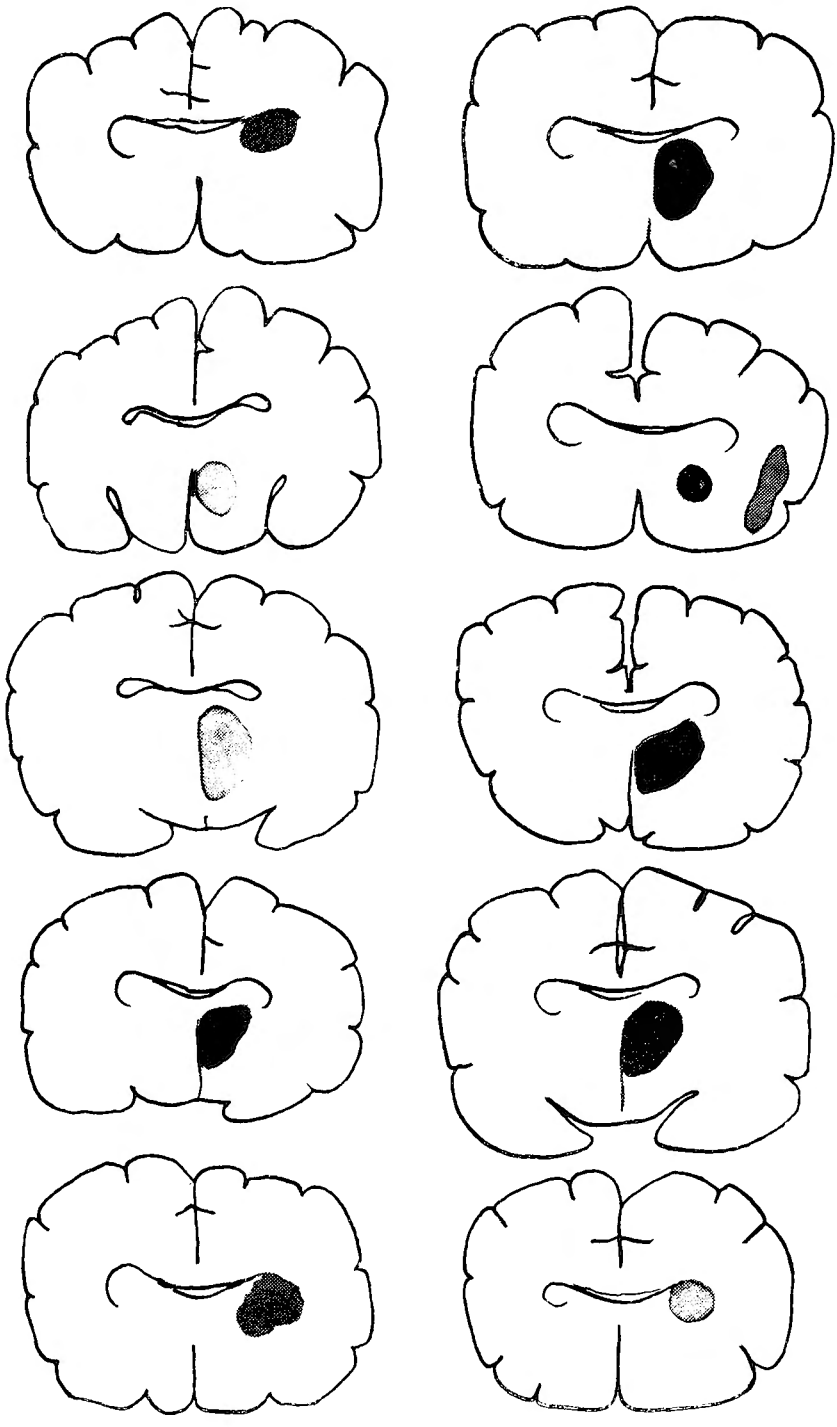

Fig. 1-a showing proximal clipping group

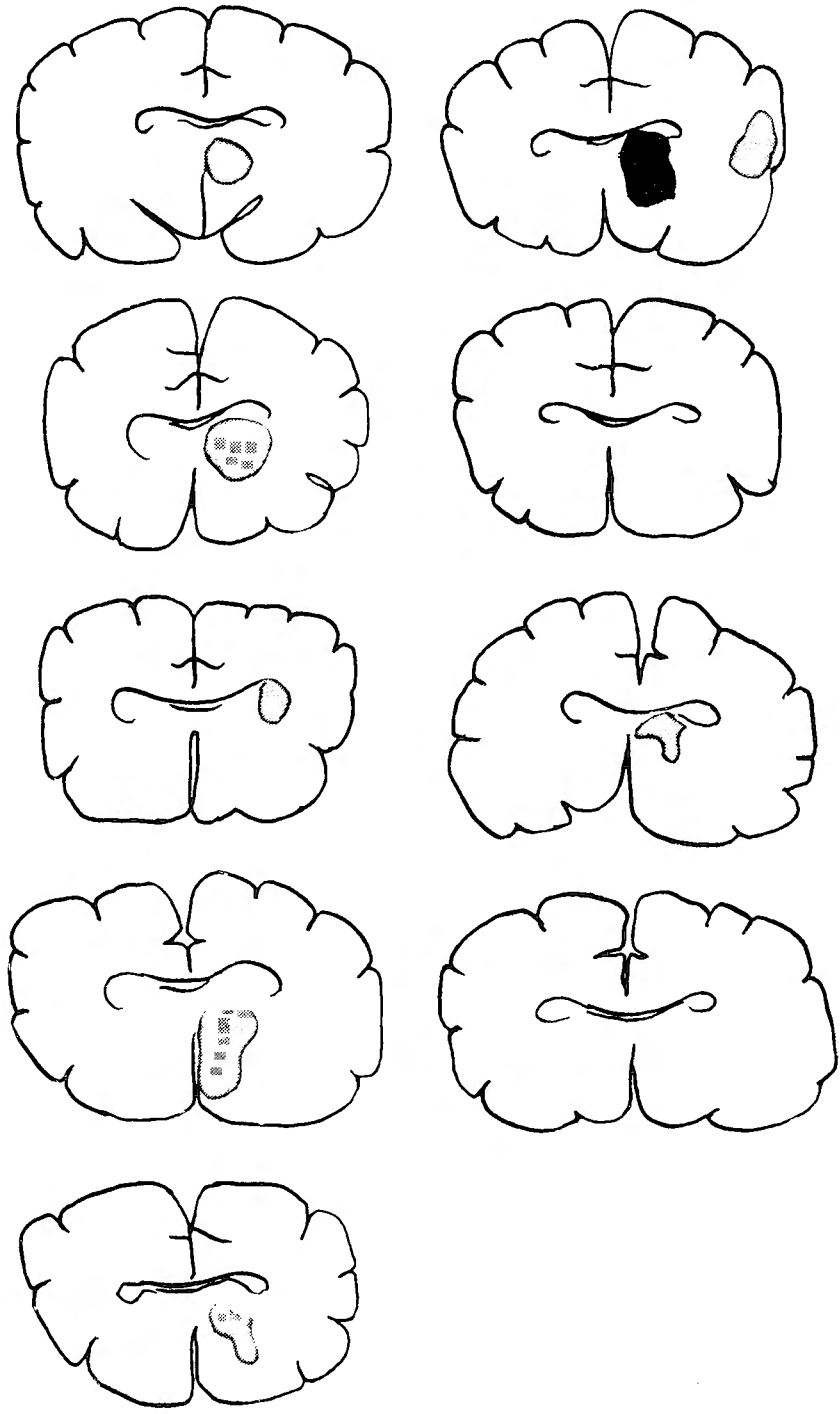

Fig. 1-b showing distal clipping group

Table 1. Showing the results of m.c.a. clipping in the different position

sites of occl. \ lesions	anemic	hemorrhagic	no change
proximal	3	7	0
distal	6	1	2

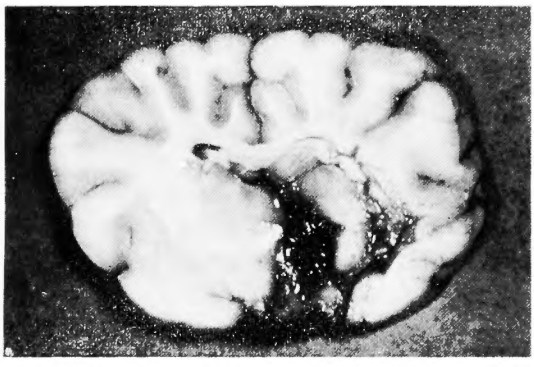

Fig. 2-a showing a hemorrhagic infarction

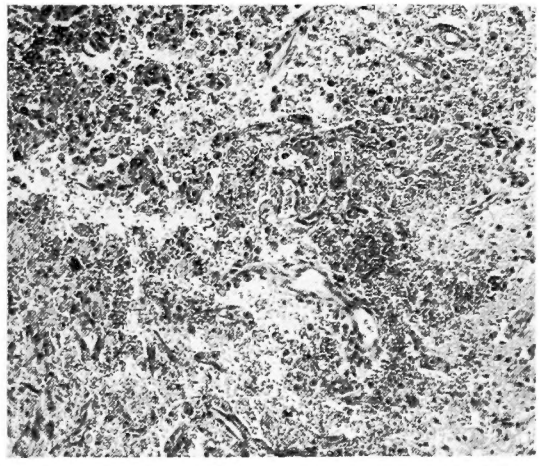

Fig. 2-b showing a microscopic appearance of a hemorrhagic infarction

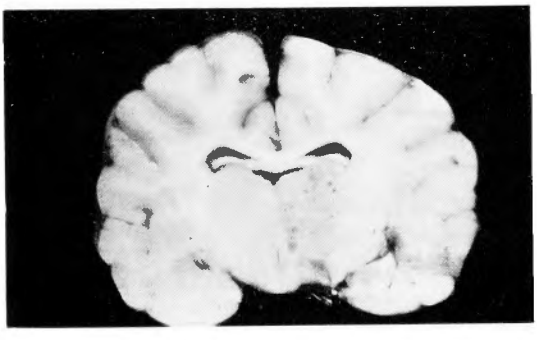

Fig. 3-a showing an anemic infarction

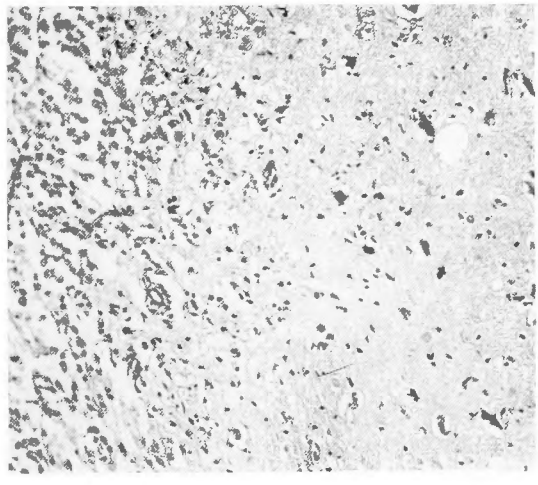

Fig 3-b showing a microscopic appearance of an anemic infarction

gic infarction and Fig. 2-b its microscopical findings in which the large amounts of extravasated red cells are visible, and the nerve cells and glial elements are severely destroyed, and blood vessels are dilated and congested in and around the infarcts. Fig. 3-a shows an ischemic infarction and Fig. 3-b its microscopical findings in which the brain tissue is severely destroyed and the infarcted foci are invaded by numerous fat granule cells, polymorphonuclear cells, but vascular dilatation or red cell stasis is scanty and extravasation of red cells is not visible.

COMMENT

GLOBUS et al[8]. attributed the hemorrhagic lesions to the effect of neosinephrine or sustained hypertension upon previously infarcted cerebral tissue. However, the above-mentioned results demonstrated that hemorrhagic infarctions limited to the thalamus and basal ganglia could be produced in dogs without the administration of neosinephrine, and also that hemorrhagic infarctions seemed to be more easily produced when the occluding clip on the middle cerebral artery proximal to the origin of the perforating branches was made rather than in cases with distal clipping. These results agree with those obtained by HAIN and his collaborators[9], who emphasized that the hemorrhagic or non-hemorrhagic infarctions could be resulted mainly by the site of occlusion of the middle cerebral artery.

However, there still remain several problems which should be elucidated before HAIN's conclusions are accepted. These consist of (1) hemorrhagic foci are found even in the distal clipping group., (2) some differences in severity of hemorrhage are found even in cases with hemorrhagic infarctions, although the systemic blood pressure and other conditions are kept almost constant throughout the procedure., and (3) some cases show the intermediate type infarction between the hemorrhagic and non-hemorrhagic macroscopically as well as microscopically even in the proximal clipping group.

PART II

PART I showed that hemorrhagic foci were more easily produced in cases with the proximal clipping of the middle cerebral artery, rather than in cases with the distal clipping. Such a difference caused by the site of occlusion of the middle cerebral artery seems to be due to the grade of the collateral blood flow which supplies the basal ganglia and thalamus.

In PART II, therefore, the changes of the intraluminal pressure of the middle cerebral artery was measured following occlusion of the different portions of the middle cerebral artery to know the grade of the collateral circulation.

METHODS

a) In two dogs, a small polyvinyl catheter, ca. 0.5mm in diameter, was introduced into the middle cerebral artery from the point of its origin toward the distal, remaining the tip of the inserted catheter (totally occluding the lumen of the vessel) in the proximal portion of the origin of the perforating branches with the care of causing no obvious spasm (Fig. 4-a). The catheter was attached to strain gauge and the intraluminal pressure of the middle cerebral artery was recorded.

b) In two dogs, the catheter was inserted into the middle cerebral artery from one of the major cortical branches toward the proximal and the tip of its occluding catheter was remained in the distal portion of the origin of the perforating branches. The intraluminal pressure was measured in the same way as that of a) (Fig. 4-b). In both groups, systemic blood pressure was recorded simultaneously.

RESULTS

Results are shown in Fig. 4-a and Fig. 4-b. In the proximal clipping group, the

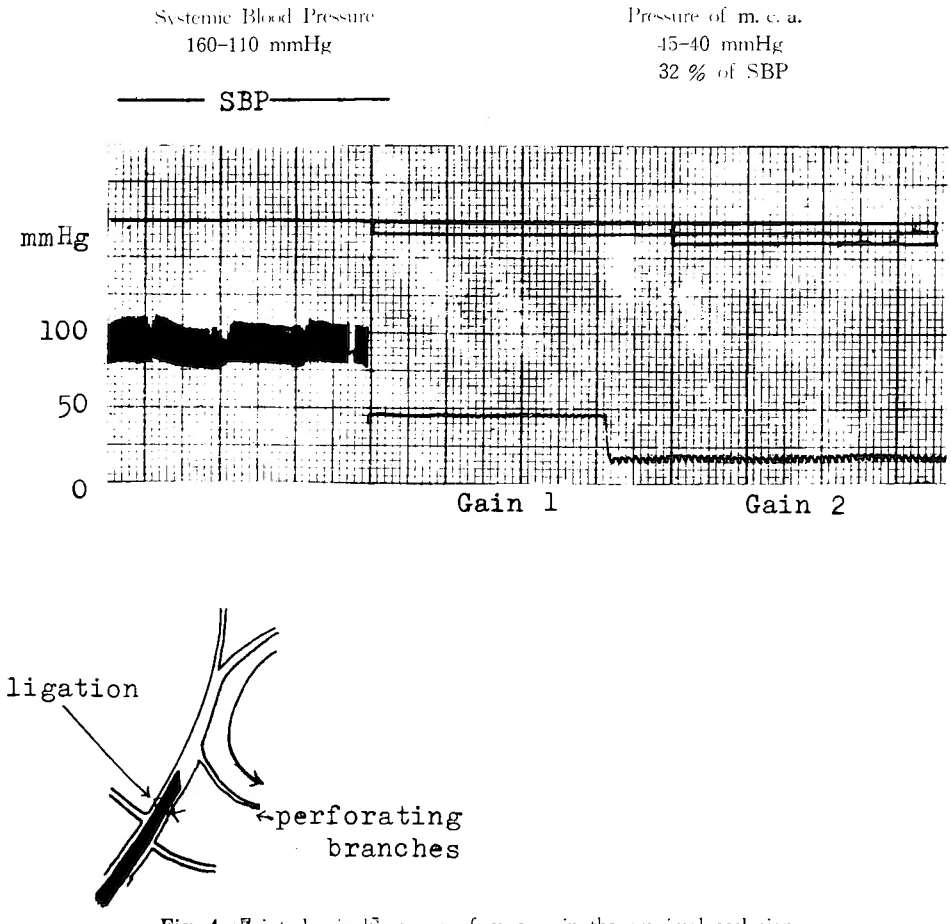

Fig. 4-a Intraluminal pressure of m. c. a. in the proximal occlusion

mean pressure of the middle cerebral artery was 43mm Hg which corresponded to only 32% of the systemic blood pressure. On the other hand, in the distal clipping group, the mean intraluminal pressure was 98mmHg which corresponded to 82 % of the systemic blood pressure.

COMMENT

As shown in above-mentioned results, the remarkable difference of the intraluminal pressure of the middle cerebral artery was found between cases occluded distally and those occluded proximally; the former was 98mm Hg (82% of the systemic blood pressure) and the latter 43mm Hg (32 % of the systemic blood pressure). This result shows that the reversed flow exists even after the proximal clipping has been made, and also that the intraluminal pressure caused by such a reversed flow is extremely low about only 43 mm Hg. According to some authors[12)13)16)] when the mean arterial pressure drops below 60-70mm Hg which they call the critical level and lasts for about more than one hour, a corresponding decrease in the blood flow occurs and cerebral infarcted foci develop in human cases. The fact that all ten cases with the proximal clipping group developed he-

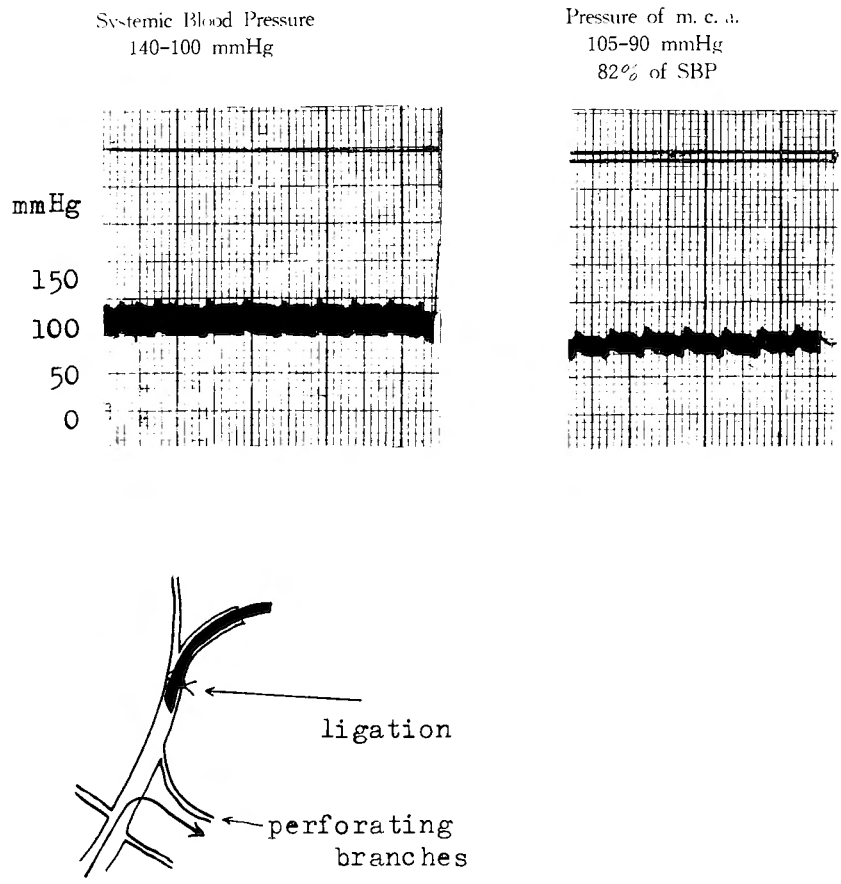

Fig. 4-b intraluminal pr. of m. c. a. in the distal occlusion

morrhagic and anemic infarctions may be explained by the drop below the critical level of the intraluminal pressure. On the other hand, as shown in PART I, the cerebral infarction occurred in seven out of nine in the distal occluding group, in which the mean intraluminal pressure was kept in 98mm Hg which is higher than the so-called critical level of blood pressure. At any rate, the occurrence of the infarction may be explained by the change of the intraluminal pressure following occlusion of the middle cerebral artery, but the difference of the nature of the infarction, hemorrhagic or anemic, can not be explained only on the basis of the change of the pressure.

PART III

As shown in PART I, there were some differences in hemorrhagic tendency of cerebral infarction even by the same clipping methods of the middle cerebral artery. Moreover, it was noted that diapedesis was almost always stopped abruptly at the limits of the white matter as shown in Fig. 2-a. In this regard, the estimations of tissue plasminogen activators in the softening area as well as in the gray and white matter was attempted.

The significance of the tissue plasminogen activator is at present unknown. However, some interrelation is considered to exist between the factors involved in the formation of fibrin deposits and those involved in their resolution. If fibrin deposits are continuously being formed in the organism as the result of the liberation of thromboplastic agents from traumatized tissues, resolution of these deposits would be of importance[1]. Accordingly, ASTRUP (1954)[2] considered that local liberation of the tissue activator could bring about resolution, and that the tissue activator could be the cause of local hemorrhage in the tissues. This assumption is in accordance with the fact that the adrenal gland and the prostate have a large concentration of activator and hemorrhage into these organs is often observed.

Present attempts were tried to differentiate the concentration of the activator in hemorrhagic and anemic infarction and, furthermore, the gray and white matter.

MATERIALS AND METHODS

The plasminogen activator was estimated on standard fibrin plates with 0.1% fibrinogen by the methods reported by ASTRUP and MÜLLERTZ (1952)[3]. The fresh tissue was homogenized with 2M-KSCN and then the homogenates were centrifuged. The aliquot of the supernatants was taken and 1N- Hcl was added to make them acid (pH 1). After the solutions were centrifuged again, the sediment was taken and neutralized by $NaHCO_3$. The aliquots of them were dropped onto the standard fibrin plates. The activator activities were recorded as the diameter products in sq. mm. of the lyzed zone after 20 hours at 37 degree C. The tissues taken from many different organs in dogs were, as a control, examined, then softening tissues caused by occlusion of the middle cerebral artery were examined, too.

RESULTS

(1) Fibrinolytic activities of each organ were shown in Fig. 5, in which mean values of the concentration of activators were also given.

(2) Fig. 6 showed the difference of fibrinolytic activities between the hemorrhagic and the anemic infarction of the basal ganglia or the cortex.

(3) Fig. 7 showed the differences between the cerebellum, pons, and the cerebrum. (cortex ; white matter, basal ganglia ; gray matter)

COMMENT

ASTRUP and STAGE found that the tissue activator could be made water soluble by treatment with potassium thiocyanate[4]. Moreover, ASTRUP and STERNDORFF, (1956)[5] reported that as the tissue activator is stable at acid reaction, the tissue activator could be easily separated from the labile plasminogen activator present in blood. Thus, the methods for the quantitative estimation of the tissue activator was established.

The results examined from the different animal tissues which were prepared by the method of ASTRUP and ALBRECHTSEN are almost the same as those reported by ALBRECHTSEN[1], as shown in Fig. 5. Thus, there is no question about the difference of fibrinolytic activity in various organs. However, no remarkable differences in the quantity of activators

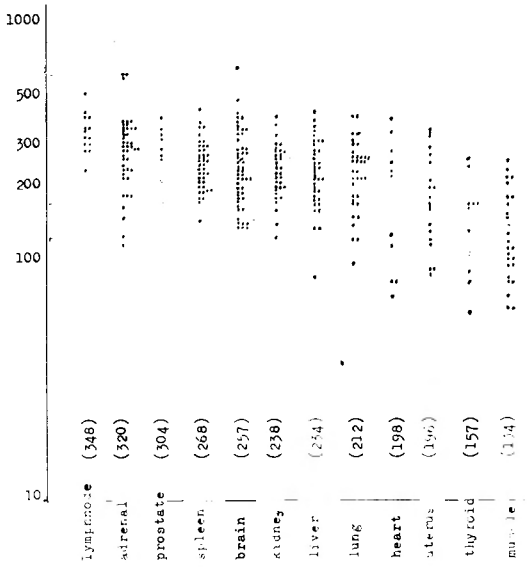

Fig. 5 showing the concentration of tissue plasminogen activator in the different canine tissues

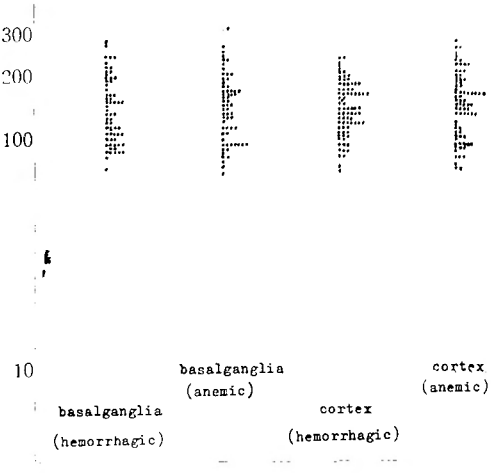

Fig. 6 showing the difference of concentration of tissue activator between the hemorrhagic and anemic infarction of the basal ganglia or the cortex.

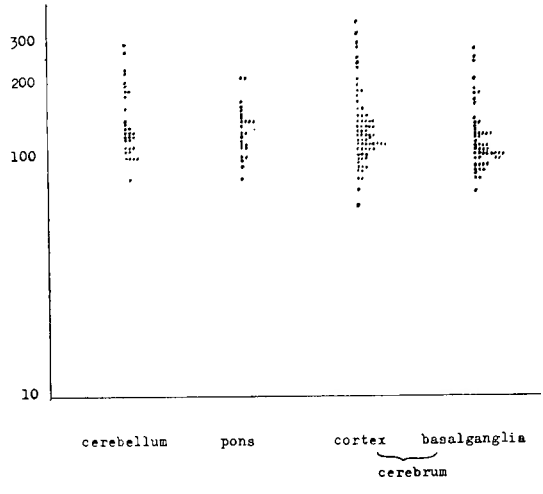

Fig. 7 showing the difference of concentration of tissue activators between the cerebellum, pons, and the cerebrum.

were found between the hemorrhagic and non-hemorrhagic portions and also between the white and gray matter. These results show that this method of the tissue plasminogen activator assay is not adequate to differentiate quantitatively the grade of hemorrhage which occurred into the infarcted foci as well as the quantity of plasminogen activators of the gray and white matters.

PART IV

In PART IV, the vital staining methods were used to differentiate more clearly the hemorrhagic or non-hemorrhagic infarctions.

METHODS

The left anterior cerebral artery was clipped at its origin from the internal carotid artery and also at the site of its distal portion (after branching the HEUBNER's artery), and the posterior cerebral artery (A. thalamo-perforata) and posterior communicating artery were also clipped at their origins. After these clippings were performed, the middle cerebral artery was occluded proximally or distally to the origin of the perforating branches. Soon after the operation, the trypan blue, 10mg per kg body weight, was administered intravenously. The dogs were sacrificed 24 hours after the operation. The brains were fixed in 10% formalin saline for about one week and were sectioned and examined macroscopically.

RESULTS

In all three cases in which the clipping proximal to the origin of perforating branches was carried out, the area restricted to the thalamus and basal ganglia was stained blue. On the other hand, in two out of three cases with clipping distal to the perforating branches, almost the same area as those of the proximal clipping group was also stained blue in colour, although the staining was observed more densely in the proximal clipping group. Fig. 8-a showed the result of the proximal clipping and Fig. 8-b showed the result of the distal clipping.

COMMENT

It is well known that the central nervous system is not vitally stained under normal conditions with the exception of the choroidal plexuses, the meninges, the wall of the cerebral capillaries and a number of special regions of the brain. In the pathological conditions, however, the brain can be stained by dyes, such as trypan blue and diiodofluorescein.

In PART IV, the cerebral arteries in the left side were ligated except the left middle cerebral artery to exclude the collateral circulations which seemed to participate in supplying the area of the basal ganglia as well as the thalamus. The thalamus and basal ganglia were stained selectively as shown in Fig. 8.

Thus, it may be considered that the lesions restricted to the basal ganglia or the thalamus, which occurred in this experiment, are due to the damage of the vessels and the increase of permeability of vessel walls by local anoxia.

PART V

In PART IV, using vital staining method, the localized changes of the vascular permeability was revealed. However, this method is not still suitable to differentiate quantitatively the grade of hemorrhage between hemorrhagic and non-hemorrhagic infarction although the difference of the staining is visible to some extent. In PART V, therefore,

the Radioactive Iodinated Serum Albumin (abbreviated to RISA herein) was used in cases prepared by the same clipping method.

METHODS

Soon after the same method of clipping was carried out as that of PART IV, RISA labelled with iodine 131 was administered by a femoral vein in doses of 5 microcuries per kg body weight. Lugol's solution was not administered in this procedure. The animals were sacrificed 20 hours after the injection and then the physiological saline was perfused from the main cervical arteries to wash out the residual blood of the brain. After removing the brain, it was frozen by dry ice and sectioned. The areas of brain tissue from the opposite hemisphere were also removed. The tissues were weighed in the wet state and homogenized with distilled water and then the aliquots of the homogenates were placed in dry, weighed metal capsules for drying and assay. The dried films were assayed for radioactive content by Geiger-Müller counter (Mica window type B-1B, Shimazu Co.). The background count was fairly stable throughout this experiment and it ranged between 30-36 cpm.

RESULTS

Table 2 showed the values of radioactivities of the infarction by the distal clipping

Table 2. DISTAL (Occlusion of the m. c. a. distal to the perforating branches)

No. of cases \ cpm		op. side	control side	ratio
R	1	786.6	189.6	6.1
R	2	3,126.0	252.0	12.4
R	9	2,046.1	168.2	12.2
R	11	996.2	111.3	8.7
R	13	3,189.0	292.1	10.8
R	17	2,028.2	269.9	7.5
R	18	1,828.0	555.1	8.7
R	21	1,104.1	75.2	11.7
m. v.				10.1

m. v. : mean value, $\sigma = 2.7$, $\sigma_m = 1.0$

Table 3. PROXIMAL (Occlusion of the m. c. a. proximal to the perforating branches)

No. of cases \ cpm		op. side	control side	ratio
R	4	1,551.0	102.0	15.2
R	5	2,196.0	120.0	18.3
R	8	3,141.0	228.0	13.8
R	10	8,211.1	353.8	23.2
R	12	6,001.0	250.2	23.9
R	16	1,278.1	132.2	9.7
R	20	512.0	57.0	9.5
R	21	160.0	15.0	10.6
R	35	2,742.0	162.0	16.9
m. v.				15.7

m. v. : mean value, $\sigma = 5.1$, $\sigma_m = 1.8$

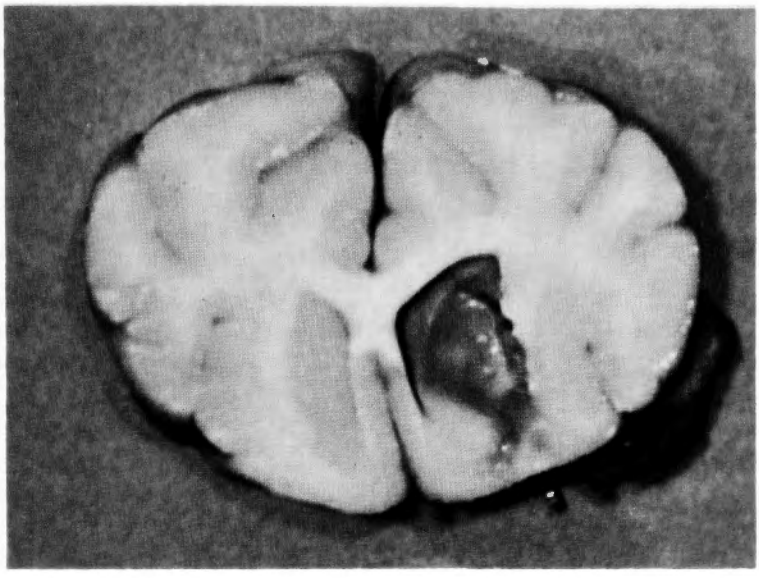

Fig. 8-a showing the staining by the proximal clipping

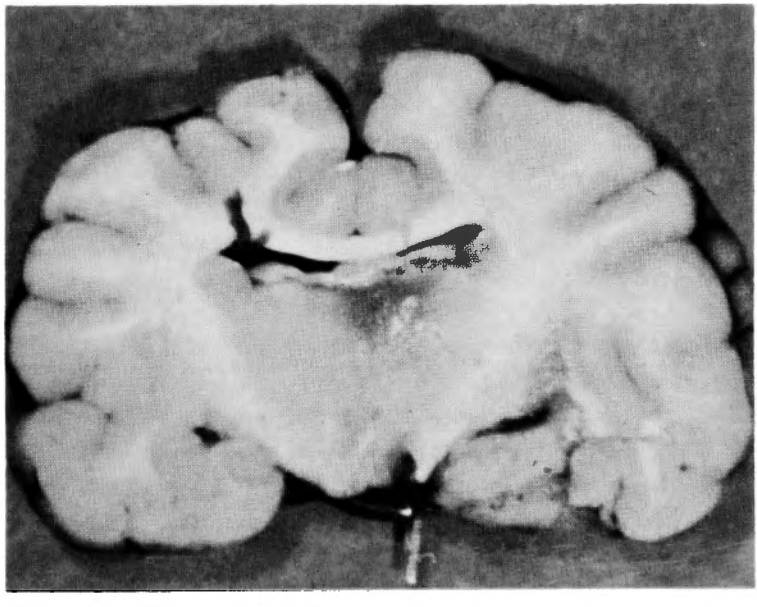

Fig. 8-b showing the staining by the distal clipping

of the middle cerebral artery and those of the opposite control brain tissue.

Table III showed the values of radioactivities of the infarction by the proximal clipping of the middle cerebral artery. The mean value of the ratio of the distal clipping group was 10.1 ± 1.0 and that of the proximal clipping group was 15.7 ± 1.8. There were significant differences between the two groups.

COMMENT

The brain scarcely shows under normal conditions increase of radioactivity after intravenous administration of isotope with the exception of special regions, such as choroidal plexuses, the wall of the capillaries and meninges, etc[6]. However, cerebral blood vessels become permeable to the protein molecules under pathological conditions, such as damage of the vessel walls or local anoxia, etc.

In this experiment, the concentration of RISA in the blood stream remains almost constant around 20 hours after its venous administration[14]. With the methods of perfusion technique, most of the residual blood could be washed out. The vessels of the basal ganglia were so small in caliber that the remaining blood after perfusion was thought to be negligible..

When the middle cerebral artery was occluded distally to the origin of the perforating branches, the values of radioactivity of the lesions were 10.1 times the values of the opposite hemisphere, whereas in cases ligated proximally, the values of the infarcted foci were 15.7 times the values of the control side. On the other hand, when the values of radioactivity per gm brain tissue of the lesions were counted by dry weight assays, the ratio to the control side became somewhat larger than by the wet weight assays, but the rate of this increment remained almost constant in the same clipping group. This fact indicated that the edema of the lesions held almost the same degree according to the same clipping methods. Therefore, it may be considered that the ratio in values of the proximal clipping group is larger than that of the distal clipping group, showing the significant differences in both groups statistically.

PART VI

The clinical use of the fibrinolytic enzymes has recently been investigated and the feasibility of this treatment has been proved in cases with thromboembolic diseases, such as peripheral, pulmonary and coronary vasular occlusion[7][15][18]. In cerebral vascular occlusive diseases, however, the fibrinolytic treatment has not yet generally been accepted because of its difficulty in clinical differentiation between cerebral vascular occlusions and cerebral hemorrhage and/or for the fear of hemorrhage into the infarcted foci.

On the other hand, J. HANDA and K. YOSHIDA in our laboratory, reported that cerebral softenings experimentally produced might be prevented by the intravenous injection of the fibrinolytic enzymes without inducing or intensifying further intracerebral hemorrhage.

The following investigation was carried out to reexamine this fact quantitatively by the use of radioactive agents.

METHODS

After clipping the proximal portion of the middle cerebral artery, as done in PART V, 5 microcuries per kg body weight of RISA was injected. In 100-200 ml. of physiological saline solution Streptokinase 4,000 units per kg body weight and human plasma of 0.5 ml. per kg body weight were dissolved[20]. The solution was kept 37 degree C for about 10 min. before its use and administered by femoral vein taking 1 hour for perfusion.

In group (a), this treatment was performed soon after the clipping operation and in group (b), 48 hours after the operation. In group (c), RISA was administered without any fibrinolytic treatments 48 hours after the operation. And the fibrinolytic activity was measured by thrombelastograph or by euglobulin lysis time for a few hours. The dogs were sacrificed 20 hours after the operation and the brains were sectioned. Radioactivities of the area of the lesions and of the opposite control side were counted in the same way.

RESULTS

The fibrinolytic activity during the treatment is shown in Table VII, Fig. 9. The ratio of radioactivities in this group was shown in Table IV, V and VI. No intensifying of hemorrhages was found in the infarcted lesions macroscopically in the group (a) which was treated soon after the operation.

Table 4. PROXIMAL with plasmin immediately after clipping

No. of cases	cpm op. side	control side	ratio
R 25	516.0	49.8	10.4
R 33	2,397.2	230.5	10.4
R 37	768.0	96.0	8.0
R 38	2,430.0	582.1	4.2
R 39	1,386.0	95.4	14.5
R 40	1,024.0	78.0	13.1
R 41	966.0	150.0	6.4
m. v.			9.6

m. v. : mean value, $\sigma = 3.6$, $\sigma_m = 1.5$

Table 5. PROXIMAL with plasmin 48 hrs. after clipping

No. of cases	cpm op. side	control side	ratio
R 42	927.0	90.0	10.8
R 43	588.0	30.1	19.6**
R 46	126.2	60.0	2.1*
R 47	471.1	60.3	7.9
R 48	714.3	42.0	17.0
R 55	1,504.0	77.8	19.3**
R 56	951.0	168.2	5.7
m. v.			11.8

m. v. : mean value, $\sigma = 6.7$, $\sigma_m = 2.7$

* showing small anemic infarction,

** somewhat hemorrhagic infarction

Table 6 PROXIMAL with RISA 48 hrs. after clipping

No. of cases	cpm	op. side	control side	ratio
R	57	941.1	91.2	10.3
R	58	601.2	30.9	19.5**
R	60	778.0	95.2	8.2
R	61	172.0	14.8	11.7
R	63	721.3	41.0	17.7**
m. v.				13.5

m. v. : mean value, $\sigma = 1.6$, $\sigma_m = 2.3$
** showing hemorrhagic infarction.

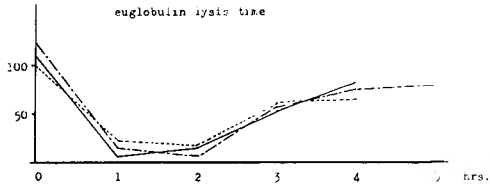

Table 7 showing changes in fibrinolytic activity in animals treated with plasmin The euglobulin lysis time was shorted to 10 min. or 70 min. in 1 to 3 hours after the administration of SK.

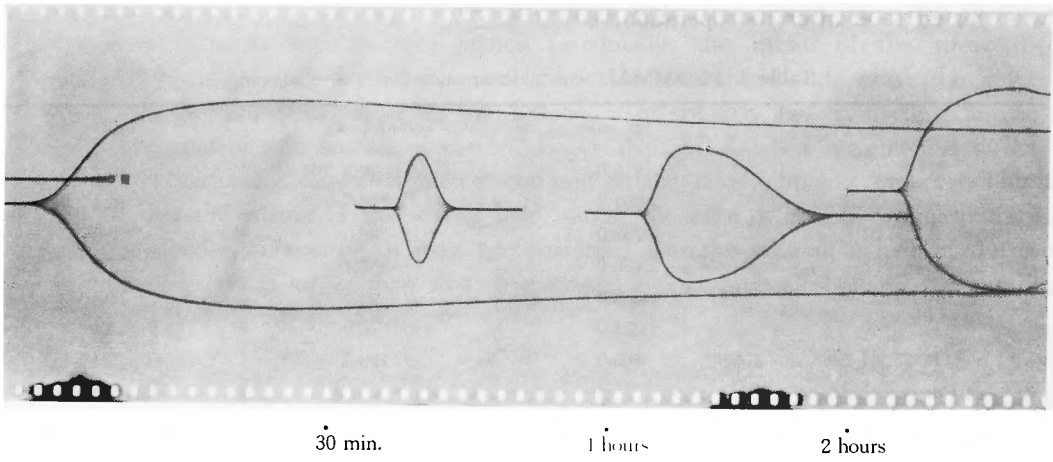

Fig. 9 This TEG showing the shortage of L and MA.

As shown in Table 4, isotope uptakes of the lesions were remarkably reduced in the group (a) (9.6 ± 1.5) treated by plasmin, as compared with those of the group with no treatment (15.7 ± 1.8), (Table 3, 4). This fact indicates that there are significant differences in both groups statistically. As shown in Table 5, the mean value of the ratio of the group (b) was 11.8 ± 2.7 and those of the group (c) without any treatment was 13.5 ± 2.3. In Table 5 * shows small anemic infarction and ** shows somewhat hemorrhagic infarction. There are no significant differences between the group (b) and the group (c) which has no fibrinolytic treatment.

COMMENT

It is well known that when fibrinolytic enzymes are used in clinical cases with cere-

brovascular disease, (1) the dosage and mode of applications, (2) timing of administration, etc. are of the most importance. On these problems, HANDA and YOSHIDA in our laboratory studied experimentally in dogs in which localized cerebral softening foci and carotid thrombosis were produced and they reported that significant decrease in size of the infarct as well as no intesifying of hemorrhage in the foci were found when treated in the early stage, usually within 48 hours.

In my experiment, the ratios of the radioactivity content were found to be much smaller in dogs treated by fibrinolytic enzymes just after the procedure, as compared with those of the group with no treatment, as shown in Table 3, 4.

However, no significant difference was found statistically between the group treated with fibrinolytic enzymes 48 hours later and the group without any treatment, (Table 5, 6) although the ratio of the radioactivity in the former group seemed to be a little smaller in the mean values.

DISCUSSION

I. Pathogenesis of hemorrhagic infarction

Numerous concepts of the pathogenesis of hemorrhagic infarction have been delivered. These include ; diminished arterial blood flow plus venous stasis (EVANS and SCHEINKER, 1943), reduction in the arterial blood flow with subsequent stasis, diapedesis and extravasation (FAZIO, 1949), temporary embolic arterial occlusion with subsequent dislodgement of the embolus (FISHER and ADAMS, 1950), dependence on the site of occlusion of the middle cerebral artery (HAIN et al. 1952), and the conditions of the previous state of the vascular bed, the degree of parenchymal support, the availability of collateral flow and the vascular pressure (GLOBUS and EPSTEIN, 1953), etc.

These facts seem to demonstrate that at least two factors are requisite for the production of the hemorrhagic infarction. The first is the sufficient alteration in the permeability of the vessel wall to permit the escape of blood into the tissue. The second is the presence of a sufficient volume of blood which flows through the vessels distal to the site of occlusion enough to produce a hemorrhagic softening.

It is well known that the increase in the permeability of the vessel wall is caused by several factors, such as the increase in vascular resistance, the slowing of the blood flow, accumulation of the vasodilatatory substances[17] and fall of the blood pressure below the critical level of 60-70mm Hg[16].

When a vessel is occluded, the blood flow distal to the occlusion is usually slowed down and the blood pressure is also dropped according to the grade of the collateral circulations. The slower the blood flow becomes and the more severer the fall of the blood pressure occurs the more remarkable increase results in the vascular resistance and accumulation of the vasodilatatory substance.

In my experiments, the intraluminal pressure of the middle cerebral artery was dropped in 98mm Hg (82% of the systemic blood pressure) when clipped distally and in 43 mm Hg (32% of the systemic blood pressure) when clipped proximally. Moreover, the ratio of radioactive contents of RISA was 15.7 when clipped proximally, whereas 10.1 when clipped distally. These facts demonstrate that a remarkable increase in the permea-

bility of the vessel wall may easily develop in the proximal clipping group rather than in the distal clipping group.

As to the presence of a sufficient volume of blood enough to produce a hemorrhagic foci in the area distal to the site of occlusion, it is well known that abundant collateral channels, such as leptomeningeal, internal and external ophthalmic arteries exist in dogs. Moreover, even if the middle cerebral artery is occluded, substantial blood flow continues in its fields of the supply usually through collaterals from the anterior cerebral artery and posterior cerebral artery and also from the other hemisphere (SYMON)[19] Fig. 10. Moreover, as shown in Fig. 4-a, the reversed direction of the blood flow exists even if the middle cerebral artery was occluded in the proximal portion.

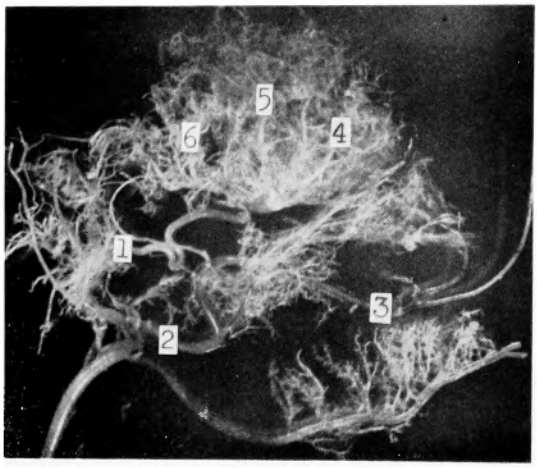

Fig 10-a showing angioarchitecture of canine brain and also showing abundant collateral channels 1 ; internal carotid artery 2 ; external carotid artery, 3 ; ophthalmic artery, 4 ; ant. cerebral artery, 5 ; middle cerebral artery, and 6 ; post. cerebral artery

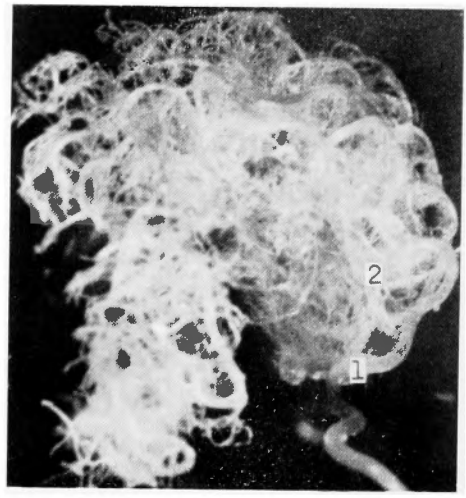

Fig. 10-b showing 1 ; middle cerebral artery 2 ; perforating ganglionic branches

Taking these facts into considerations, it may be conceivable that when clipped proximally, the reversed blood flow to supply the basal ganglia is not sufficient to maintain both the cerebral parenchyma and the vessel walls, but is adequate to make the lesions hemorrhagic owing to the increased permeability of the vessel walls, while, when clipped distally, the diapedesis of the blood elements may be prevented.

Quatitative methods using the radioisotope assay also demonstrates these facts.

II. The application of fibrinolytic treatments for canine cerebral infarction

The values of radioactivities in the lesions undertaken immediately after the production of the infarct were reduced remarkably. Moreover, no intensifying of hemorrhage was noted. These results demonstrate clearly that fibrinolytic treatments, when used in the early stage, are effective in the treatment of the cerebral occlusive diseases.

However, no significant difference was noted in the group treated 48 hours after the production of the lesions, although no intensifying of hemorrhage was observed.

Thus, these results indicated that good results might be expected when fibrinolytic enzymes were used within 48 hours after the procedure, especially in the early stage, in cases with the cerebral occlusive diseases.

SUMMARY AND CONCLUSION

The mechanism of the production of different types of cerebral vascular lesions, especially the hemorrhagic infarction, has not yet clearly demonstrated.

The present study, therefore, was intended in dogs to elucidate the pathogenesis of hemorrhagic infarction, and thereafter, the effects of the fibrinolytic treatments on the cerebral infarction were investigated.

PART I : The character of the lesions was generally depending on the site of occlusion of the middle cerebral artery, when the systemic blood pressure was kept almost constant throughout the procedure.

In the proximal clipping group, seven out of ten cases were hemorrhagic, whereas in the distal clipping group, six out of nine dogs were anemic.

PART II : The changes of the intraluminal pressure was recorded. In the proximal clipping group, the mean pressure was 43mm Hg in a reversed direction of flow, whereas in the distal clipping group, the pressure was 98mm Hg.

PART III : The tissue plasminogen activator activity was estimated to know the differences of the property of the hemorrhagic or non-hemorrhagic infarction quantitatively, resulting in impossibility to find out remarkable differences between the two distinct infarctions and between the gray and white matter.

In PART IV, the attempts to make the difference of the hemorrhagic or anemic infarctions more clearly with the aid of vital staining methods were undertaken. The basal ganglia and the thalamus were localizedly stained blue, and the tone of the colour was observed stronger in the proximal clipping group.

Then, in PART V, to differentiate the property of the infarction more strictly, the quantitative method of radioisotope assay was performed. In the proximal clipping group, the values of radioactivity contents demonstrated about 15.7 times the values of the opposite control region, whereas in the distal clipping group, about 10.1 times the values of the control side, showing significant difference between the two groups.

In PART VI, the effects of the application of fibrinolytic treatments to the cerebral infarction were examined using radioisotope assay. When treated in an early stage, the values of radioactivities of the treated group were reduced remarkably as compared with the non-treated group (15.7 to 9.6).

On the contrary, the values of the group treated 48 hours after the arterial occlusion showed statistically no significant difference from the values of the group with no treatments and there was found less remarkable intensifying of the hemorrhage into the lesions.

The following facts were revealed in the present study :

1) The properties of the cerebral infarction restricted to the basal ganglia or the thalamus were generally depending on the site of occlusion of the middle cerebral artery, when the systemic blood pressure was kept constant.

2) Early administration of the fibrinolytic enzymes would prevent the production of

cerebral infarction or improve the tissue damage due to the arterial occlusion, and moreover, this treatments must be performed within 48 hours after the cerebral arterial occlusion in dogs.

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〔和文抄録〕

出血性脳軟化巣の発生機序及び脳軟化巣に対する線維素溶解酵素療法の応用に関する実験的研究

京都大学医学部脳神経外科（指導：荒木千里教授）

近藤明憙

出血性脳軟化巣の発生機序に就いては，種々の議論がある．そこで，この軟化巣の性質の差を明らかにするために，RISA 等を用い定量的に之を検討した．次に軟化巣に対する，線維素溶解酵素療法の効果及びその限度につき同様検討した．

1) 先ず，全身血圧を，ほぼ一定に保ち乍ら中大脳動脈を，その穿通枝より近位側，及び遠位側で結紮した場合，前者は，10例全部に軟化巣の発生を見，うち7例が出血性，後者は9例中，7例に軟化巣，うち6例は貧血性である．

2) 中大脳動脈を穿通枝近位側，遠位側で結紮した場合，前者では，血流は皮質枝より逆行性で，血圧は，43mmHg．後者では，98mmHgである．

3) 組織アクチベーターは，各臓器間に差があり，出血性の強いと思われる臓器（副腎，前立腺等）では値が高い．然し，出血性軟化と貧血性軟化との間には，著明な差はなく，この方法では，両者を区別出来ない．

4) 前大脳動脈，後交通動脈，後大脳動脈を各々起始部で結紮した後（以下の群は同様結紮）Trypan Blue点滴静注により間脳，基底核に限局した染色性を認めるが，近位側結紮群の方が濃度が比較的濃い．然し，この方法にても，区別は不充分である．

5) RISA を用いて，軟化巣の放射能を測定した場合，中枢側結紮の場合は，その値が15.7，末梢側結紮の場合は，10.1で，両者の間には有意の差がある．

6) 中枢側結紮後，線溶療法を行つた場合，

a) 動脈結紮直後に行つたものの計測値は9.6に減少する．

b) 48時間後に行つたものの値は11.8で，無治療群13.5に比し，増加はないが，両者の間には有意の差はない．

以上の事より，次の事が結論される．即ち犬では．

1) 脳軟化巣の性質は，全身血圧に変化がなければ，主として，中大脳動脈結紮部位に関係する．之は血管透過性の変化，副血行路よりの血液供給の差によるものと思われる．

2) 脳軟化巣に対し，早期に線溶療法を行つた場合，軟化巣の発生防止，縮少に効果があると思われるが，48時間後の場合は，特に出血性の増加は認めないが，その効果は減じる．